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HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES OF LIVER TISSUE F--ETC(U)
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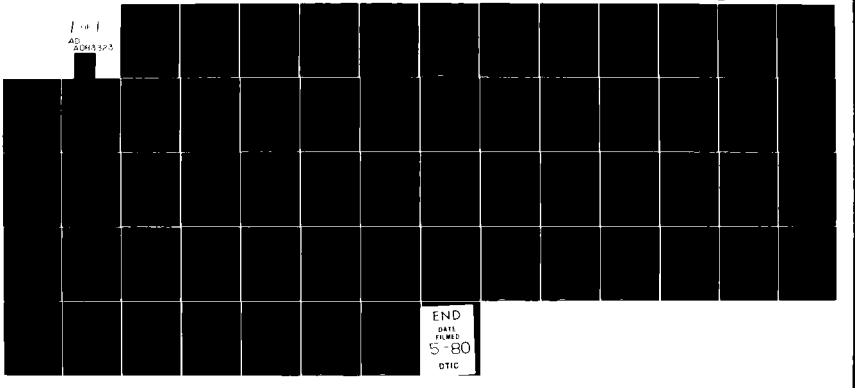

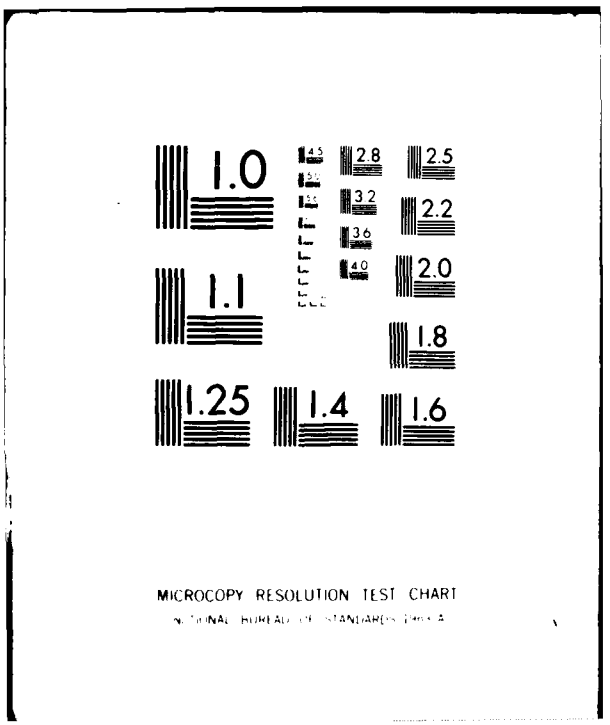
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Quantitative ultrastructural studies were conducted on liver tissue from beach mice, Peromyscus polionotus, exposed to the toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in field and laboratory environments. Hepatic tissue from 52 animals was examined for changes in smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), and mitochondria. Fifteen of 30 animals were collected from a unique military test site in northwest Florida where they had been continuously exposed to soil levels of 10 to 710 parts per trillion (ppt)

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20. Abstract (continued)

TCDD. Twelve of 22 animals were exposed 10 times in 28 days to 2.5 parts per billion (ppb) TCDD applied as a dust to their pellage. All remaining animals were from either a field control site or not exposed to TCDD in the laboratory. All tissue was examined both histopathologically and by an ultrastructural stereological technique.

The levels of TCDD in composite liver samples from mice collected in the field varied from 960 ppt for females to 1300 ppt for males, while a composite liver level of TCDD for the laboratory animals was 125 ppt. The levels of TCDD in the livers of the beach mice collected from the field substantiated bioaccumulation of TCDD but not food chain biomagnification. Although the levels of TCDD in the livers were greater than those found in the soil, TCDD was not detected in the portion of the food chain consisting of seeds. The laboratory dusting study confirmed that ingestion of TCDD can occur as a result of body contact with soil containing TCDD and subsequent grooming by the burrowing animal.

No significant histopathological or ultrastructural changes were found in hepatic parenchymal cells after long term, low level exposure to TCDD in the field, or after short term, low level exposure in the laboratory. Statistically significant differences in liver weight to body weight ratios concurrent with the absence of cellular changes following exposure to TCDD in the field is explained by the low level of exposure.

This study has demonstrated the application of the analytical technique of stereology to field studies of toxicity. The modified technique, as used in this study, may be a valuable tool for characterizing quantitative cellular responses to injury.

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MARCH 1980

HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES OF LIVER TISSUE FROM TCDD-EXPOSED BEACH MICE (*PEROMYSCUS POLIONOTUS*)

LORRIS G. COCKERHAM

ALVIN L. YOUNG

CHARLES E. THALKEN

PROJECT 2303

FJSRL-TR-80-0008

This document was prepared by the Faculty Research Division, Directorate of Chemical Sciences, Frank J. Seiler Research Laboratory, United States Air Force Academy, Colorado. The research was conducted under Project Work Unit Number 2303-F1-83, "Ultrastructural Evaluation of Tissues Removed from Animals Exposed to TCDD". Lt Colonel Lorris G. Cockerham was the Project Scientist in charge of the work.

Inquiries concerning the technical content of this document should be addressed to the Frank J. Seiler Research Laboratory (AFSC), FJSRL/NC, USAF Academy, Colorado 80840. Phone AC 303-472-2655.

This report has been reviewed by the Chief Scientist and is releasable to the National Technical Information Service (NTIS). At NTIS it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

William J. Cairney

WILLIAM J. CAIRNEY, Major, USAF, BSC
Project Scientist
Director of Research/DFCBS

Kenneth E. Siegenthaler

KENNETH E. SIEGENTHALER, Lt Colonel, USAF
Director
Directorate of Chemical Sciences

WILLIAM D. SIURU, JR., Lt Colonel, USAF
Commander

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By

Lt Colonel Lorris G. Cockerham
Major Alivin L. Young
Lt Colonel Charles E. Thalken

Department of Chemistry and Biological Sciences
USAF Academy, Colorado 80840

Prepared for:

Headquarters Air Force Logistics Command
Wright-Patterson Air Force Base, Ohio 45433

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Directorate of Chemical Sciences
Frank J. Seiler Research Laboratory
Air Force Systems Command
US Air Force Academy, Colorado 80840

PREFACE

The authors gratefully acknowledge the support of the Frank J. Seiler Research Laboratory for providing the electron microscope facility required for this project. Appreciation is also expressed to Air Force Logistics Command (AFLC/LO) for providing funds for field work and analysis of the tissues. The authors also wish to thank the Interpretive Analytical Services Division, Dow Chemical U.S.A., Midland, Michigan, and the Armed Forces Institute of Pathology, Washington, D.C., for competent and consistant analytical and histopathological support.

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INTRODUCTION

Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD) has been called the most toxic chlorine-containing compound. It may occur as a contaminant of wood preservatives, pesticides, and medical and industrial chemicals produced from chlorinated phenols (6). The acute oral LD_{50} is reported in the range from 0.6 µg TCDD/kg body weight in male guinea pigs to 115 µg TCDD/kg of body weight in rabbits (16,38). Sublethal doses have produced pathological changes in liver, spleen, intestine, thymus, lymph nodes and adrenal glands in laboratory studies (11,21,35,38). Data, however, have indicated that the liver is the major target organ for the effects of TCDD (4,11).

Rarely, if ever, in nature are men and animals subjected to massive exposures to TCDD. The few incidences of known exposure (5,20,34) are thought to have been to minute quantitites (picograms) for relatively short time periods (3-6 weeks). Most recently, the presence of TCDD as a contaminant in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) precipitated concern over the use of this herbicide in the United States (19). Under present conditions of application of 2,4,5-T herbicide, the estimated concentration in the soil would be less than one part per trillion (ppt) (19). Nevertheless, data are needed on the potential effects of low level, long-term exposure to TCDD.

The experiments reported here were designed to quantitatively assess the effects of low level exposure to TCDD on the ultrastructural hepatic morphology in animals living in the field. The goals of this study, then, were to (1) determine what ultrastructural changes occur

in hepatic parenchymal cells in response to low level, long-term exposure to TCDD in the field, (2) determine what ultrastructural changes occur in hepatic parenchymal cells in response to low level, short-term exposure to TCDD in the laboratory, (3) determine if ingestion, and hence liver accumulation, of TCDD can occur as a result of body contact and grooming and not necessarily through the food chain, and (4) demonstrate the use of stereology in the quantitative assessment of toxicity in a field environment as well as in the laboratory.

A suitable field site for this study must necessarily (1) be contaminated with significant (i.e., readily detectable) quantities of TCDD, (2) have an endemic animal population present, and (3) be isolated from human activity, yet available for investigation. A unique site in northwest Florida possessing these criteria has been reported by Young (42). In support of programs testing aerial dissemination systems, Test Area (TA) C-52A, Eglin AFB Reservation, Florida, received massive quantities of military herbicides. This approximately 2.6 km^2 test area received approximately 73,000 kg of 2,4,5-T and 76,790 kg of 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide during the period 1962-1970. Significant levels (10-710 ppt) of TCDD were found in 1973 within the top 15 cm of the test area soil.

Test Area C-52A is principally a grassy plain surrounded by a forest stand dominated by longleaf pine (Pinus palustris), sand pine (Pinus clausa), and turkey oak (Quercus laevis) (42). The portion used in the present study was a cleared area occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), woolly panicum (Panicum

lanuginosum), and low growing grasses and herbs. Of major interest in this study was an 0.4 km^2 plot located in the southern portion of the testing area. Although dissemination of herbicides at this site was discontinued after two years, it received the heaviest application. From 1962 to 1964, this site (called Grid I) received 39,547 kg of 2,4-D and 39,547 kg of 2,4,5-T. By 1969 only traces (parts per billion; ppb) of 2,4,5-T were detected (42) while TCDD was detected at significant levels in 1973 in analysis of soil samples from the top 15 cm of soil. Analysis of soil cores at 15 cm increments to a depth of 90 cm indicated no detectable TCDD (lower limit of detection was 10 ppt) below the 45 cm level. A more detailed description of TA C-52A, its history and present status, may be found in reports by Young (42) and Young, Thalken, and Ward (43). These reports are available from the Defense Documentation Center, Defense Supply Agency, Cameron Station, Alexandria, VA 22314.

The most common mammalian species reported on TA C-52A is the beach mouse, Peromyscus polionotus (30,42). This was the animal of choice for investigating long term, low dosage effects of TCDD in the field because mice have been used extensively in toxicological studies of TCDD (9,11,35,38) and thus provide known indicators of toxicity.

Concurrently with the field studies, a laboratory experiment was conducted to simulate contact of the rodent's pelage with TCDD contaminated soil. The objective of the study was to determine if ingestion of TCDD can occur in the field as a result of body contact and grooming and not necessarily through the food chain. The accumulation of TCDD in the liver would implicate grooming as a means of contact while

histopathological and ultrastructural studies of the liver would assess the effects of a low level, relatively short-term exposure to TCDD. Thus a comparison of long and short-term effects on the same species could also be accomplished.

MATERIALS AND METHODS

Soil and Seed Analysis

To establish the actual levels and the persistence of TCDD in the soil in June 1974, samples of the top 0-15 cm of soil were taken from six sites on Grid I. One of these sites was also sub-sampled at increments of 0-2.5, 2.5-5.0, 5.0-10.0, and 10.0-15.0 cm. These soil samples, along with soil samples from four designated control areas approximately 800 to 1600 meters east of Grid I, were later analyzed for TCDD concentrations.

To eliminate the food chain as an intake route for the TCDD, seed samples were taken from living plants adjacent to burrows on Grid I. These living plants were of the same species as the soil contaminated plants found in the burrows. The composite seed samples were also later analyzed for TCDD content.

Animal Description

The beach mouse is a small rodent weighing about 13 g, approximately 120 mm in length, with brown (adult) or dark gray (juvenile) fur on the back, and pale gray to white fur on the ventral region and legs (43). It may be found in old field habitats and in areas of 5% to 60% vegetative cover, preferring sandy areas.

Field work for this study was conducted in June and July 1974. Havahart traps (Havahart Traps, Dept 1, P.O. Box 551, Ossing, NY 10562), sizes 0 and 1, for small animals, were used to trap the rodents. The traps were baited with a mixture of peanut butter and oatmeal and then

randomly placed on areas of the test grid where 20% to 80% vegetative coverage was present, or near openings to mouse burrows. The four designated control areas approximately 800 to 1600 meters east of Grid I were trapped in the same manner as was Grid I.

Traps were checked daily and were moved to other locations within the test and control areas after four days failure to catch an animal. Fifty-three live mice were captured and taken to the laboratory for histopathologic examination, hepatic ultrastructural study, and chemical analysis of the tissue. Fifteen of the mice captured from Grid I were designated as treated field animals and the first 15 mice captured from the control area were designated as control field animals. The remaining 23 mice from the control area were selected to be used as subjects in a laboratory dusting study.

Laboratory Study

When it was observed that the mice spend much of their active hours grooming, another route of contact with TCDD besides the food chain was proposed. As the rodents enter and leave their burrows, they pass through the TCDD laden 15 cm of soil. This soil adheres to their pelts and as a result of the grooming habits of the beach mouse, the TCDD could be ingested in this manner. With this thought in mind, a laboratory experiment was designed to simulate a probable source of contact for the beach mouse.

Twenty-three of the beach mice captured from the designated control areas were brought into the laboratory and individually placed in separate Iso-cages (Carworth, Division of Becton, Dickinson and Co.,

New York) and maintained on laboratory chow (Ralston Purina Company, General Offices, Checkerboard Square, St Louis, MO). The 23 animals were weighed, sexed, and randomly divided (using a random numbers table) into a "control" group of 11 animals (four female and seven male) and a "test" group of 12 animals (five female and seven male). These animals were observed for two to three weeks (depending on date captured) in the laboratory to determine grooming habits and to allow for metabolic stabilization after change in diet before dusting was initiated.

The fur on the ventral thoracic and abdominal regions, sides, back and tail on each test animal was dusted with 100 mg of alumina gel containing 2.5 ppb TCDD by analysis. Control animals were dusted in the same areas but with alumina gel alone. All dusting was accomplished using a camel hair artist's brush. The 100 mg application per animal resulted in an approximate exposure of 60 mg of gel at each application per animal (based on average weight of recovered residue following dusting).

The dusting procedure was repeated every third day for a total of 10 applications during a 28 day period. On the 29th day the 22 mice (one control animal died apparently as a result of handling) were sacrificed and prepared for examination.

Animal Preparation and Examination

The 30 mice selected for the field study and the 22 mice from the laboratory study were prepared for examination using a cervical dislocation procedure to accomplish humane euthanasia. All animals were then weighed, skinned and systematically examined for gross developmental

defects such as cleft palate, cleft lip and polydactyly. Body and organ weights were recorded, internal organs were examined for gross lesions and representative sections of each tissue were placed in neutral 10% buffered formalin and processed for histopathological examination. A representative section of the liver was also processed for ultrastructural studies. All remaining liver tissues and pelts were pooled according to the study, sex and treatment, placed in glass jars, frozed and submitted for TCDD analysis.

Hepatic Ultrastructural Study

After the liver was removed from the 52 beach mice, and weighed, a section approximately one mm thick was taken from across the central lobe (Lobus centralis). This section, to be used for the ultrastructural study, was minced and transferred to containers of the primary fixative, 2% glutaralydehyde, buffered to a pH of 7.2 with Sorensen's phosphate buffer solution.

Fixation of the minced tissue was allowed to continue for two hours at 4°C prior to rinsing with buffer solution to remove any excess fixative. The small pieces of tissue were then post-fixed for one hour at 4°C with phosphate-buffered 1% osmium tetroxide. The tissue was rinsed again with buffer solution prior to dehydration with a graded series of acetone. After dehydration, the tissue was transferred directly from 100% acetone to a graded series of solutions of acetone and the embedding medium, Epon-812, and eventually to the embedding medium alone in BEEM capsules. An outline of the preparation procedure is presented in Table 1.

TABLE 1. TISSUE PREPARATION SCHEDULE TO PREPARE LIVER SECTIONS FOR ULTRASTRUCTURAL STUDY

SOLUTION	TEMP	TIME
Glutaraldehyde (2%)	4°C	2 hrs
Buffer (Phosphate)	4°C	1 hr
Buffer (Phosphate)	4°C	1 hr
Buffer (Phosphate)	4°C	1 hr
OsO_4 (1%)	4°C	1 hr
Buffer (Phosphate)	4°C	1 hr
Buffer (Phosphate)	4°C	1 hr
Buffer (Phosphate)	4°C	1 hr
30% Acetone	4°C	15 min
60% Acetone	4°C	15 min
90% Acetone	4°C	15 min
100% Acetone	4°C	15 min
100% Acetone	4°C	15 min
100% Acetone	4°C	15 min
Acetone/Epon mixture (1:1)	Rm	Overnight
Acetone/Epon mixture (1:3)	Rm	12 hrs
100% Epon mixture	Rm	Overnight
100% Epon mixture	35°C	12 hrs
100% Epon mixture	45°C	Overnight
100% Epon mixture	60°C	3 days
Cure	Rm	6 days

After the epoxy resin blocks had cured for a minimum of six days, the tissue was then sectioned with glass knives on a Sorvall "Porter-Blum" MT-2 ultramicrotome. Tissue sections of approximately 75 nm thickness were placed on uncoated copper grids and stained with uranyl acetate and lead citrate using procedures outlined by Hayat (14).

A Zeiss EM-9 electron microscope was used to examine and photograph the tissue. To insure unbiased results, a minimum of five electron micrographs were taken from radomly chosen sections. The cells selected to be photographed displayed a large cross-section of the nucleus, thereby guaranteeing that a representative cross-section of the cell was recorded.

Data for analysis was obtained from the electron micrographs through a technique known as stereology. This method of quantitative analysis of the cell ultrastructure uses morphometric procedures based on the techniques developed by Weibel et al. (39) and Weibel (40), then modified and used by Buckanan (3). This modified technique employs a method of extrapolating from areas to volumes using a system of "point counting."

A grid overlay of points to be counted was constructed by marking a grid of dots spaced five mm apart on a sheet of clear acetate. The resulting transparent grid overlay was then randomly placed over the photographic paper as the cell image (Figure 1) was printed on the paper. This produced an electron micrograph of the cell with a grid of white dots superimposed over the image (Figure 2). All of the dots lying over the mitochondria (MITO), the damaged (swollen and ruptured) mitochondria (d.MITO), the smooth endoplasmic reticulum (SER), the rough endoplasmic

reticulum (RER), and the total area of the cytoplasm were then counted visually using a push-button counter.

The volume fraction of each structure is considered to be the ratio between the point count of that structure and the total point count of the cytoplasm (24). After the volume fraction was determined for each structure of each cell photographed, the means of the volume fractions or ratios were then computed for each animal. In this manner, the ratio of mitochondrial volume to cytoplasmic volume of the hepatic parenchymal cell was determined for each animal as was the ratio of damaged mitochondrial volume to total mitochondrial volume, RER to cytoplasm, SER to cytoplasm and RER to SER. These volume fractions or ratios were used as quantitative measurements of the structures to compare the hepatic parenchymal cells from treated animals with those from control animals.

TCDD and Histopathological Analyses

To support the ultrastructural studies, analysis of the soil, seed, liver, and pelt samples for TCDD content, as well as the determination of the TCDD concentration in the alumina gel used in the dusting study, was conducted by Interpretive Analytical Services, Dow Chemical USA, Midland, MI 48640. Histopathological examination of internal organs was accomplished by the Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington DC 20305.

Statistical Analysis

The Wilcoxon Rank Sum Test was used to analyze statistically the body weight and organ weight data as well as the hepatic morphometric data. This statistical procedure is designed to test the hypothesis that

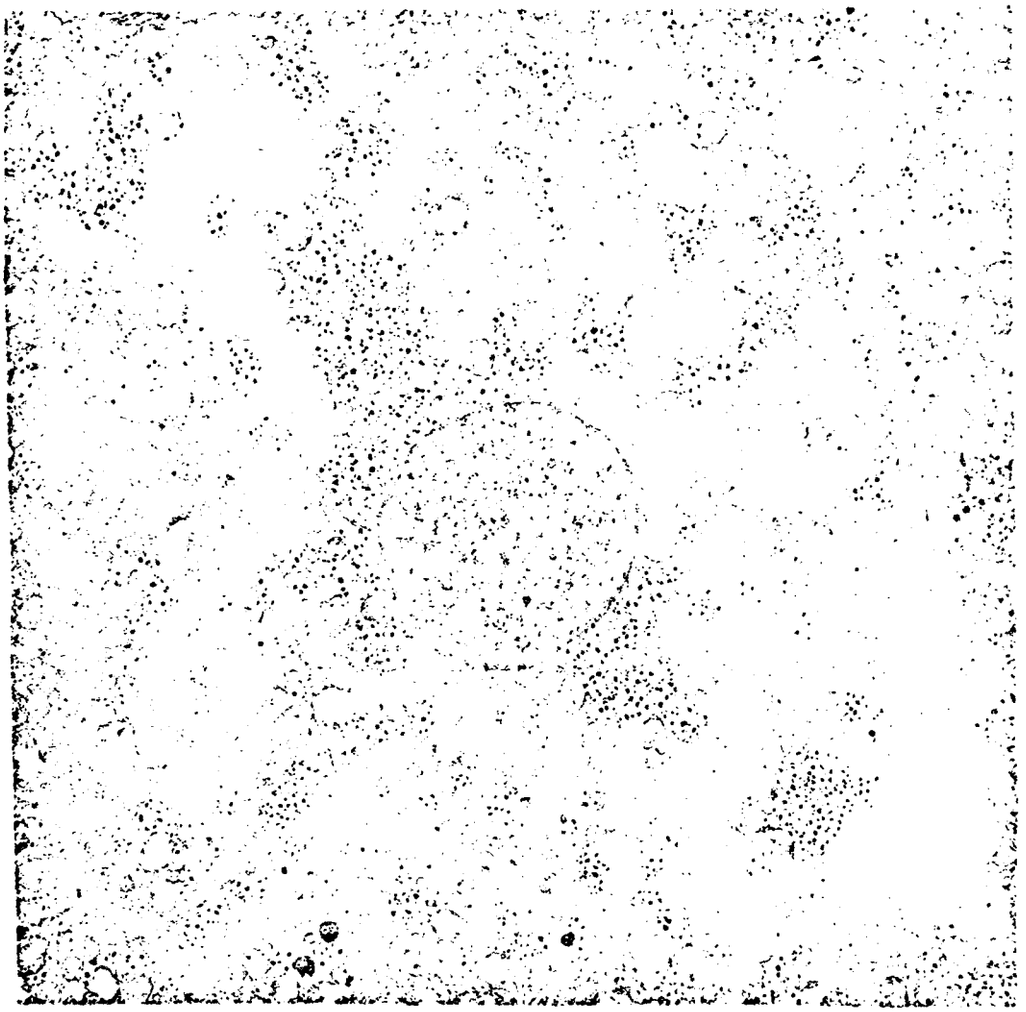

Figure 1. Hepatic Parenchymal Cell Prior to Printing with a Dot Grid Overlay. (x5726)

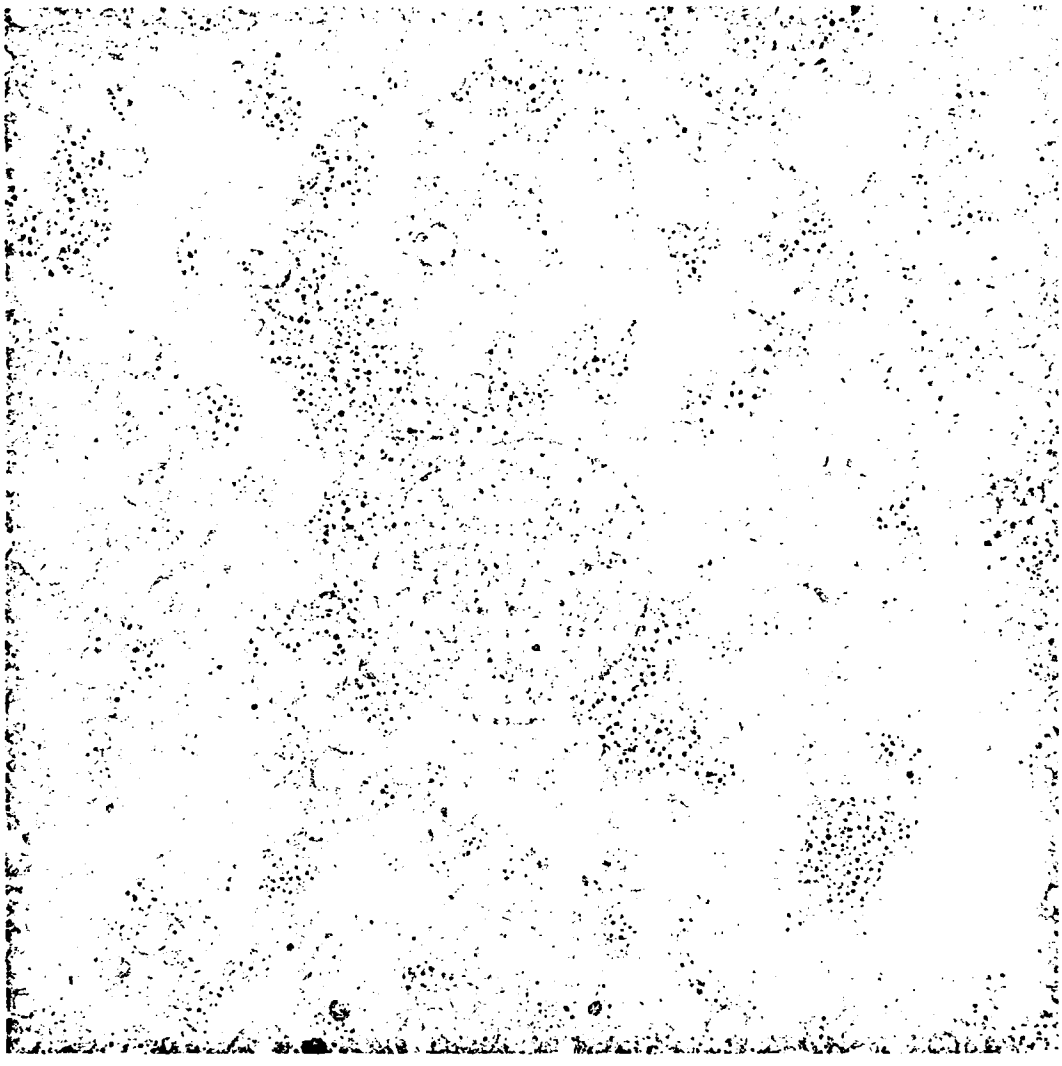

Figure 2. Hepatic Parenchymal Cell Printed with Dot Grid Overlay for Morphometric Analysis. (x5726)

that two random samples have been drawn from populations have identical distributions.

In addition, the body weight and organ weight data were statistically analyzed by Regression Analysis using linear, double logarithmic, and semi-logarithmic correlation, and by Analysis of Covariance. The Analysis of Covariance was performed using the current or final body weight as a covariate, thereby eliminating the variations in organ weight caused by variations in body weight. This method has proved superior to the analysis of relative organ weights (36).

RESULTS

Soil and Seed Analysis

There were wide fluctuations in TCDD concentrations in the mixed soil from Grid I, with TCDD concentrations of 10, 25, 70, 70, 110, and 710 ppt (Table 2). The unmixed 15 cm core, obtained from the site having 110 ppt TCDD, showed that TCDD was stratified within the top 15 cm of soil. Concentrations of 150, 160, 700, and 44 ppt TCDD were detected at depths of 0-2.5, 2.5-5.0, 5.0-10.0, and 10.0-15.0 cm, respectively. TCDD was not detected in soil samples taken from the designated control area.

No TCDD was found in any seeds taken from Grid I (minimum detection limit of one ppt TCDD).

Beach Mouse Grooming Habits

It was observed that beach mice have meticulous grooming habits, spending as much as 50% of their active hours in the process. Areas of the body that received the most grooming attention were the ventral thoracic and abdominal regions, sides, back, and tail.

Analysis of Livers and Pelts

Livers, as well as the pelts of beach mice captured from Grid I, where significantly high soil levels of TCDD were found, displayed evidence of accumulation of TCDD (Table 3). The male beach mice from Grid I displayed a hepatic TCDD level of 1300 ppt while the level for the females was 960 ppt. The pelt levels were 130 ppt and 140 ppt for the male and female mice, respectively.

TABLE 2. CONCENTRATION (PPT) OF TCDD IN SOIL FROM GRID I AND FROM THE CONTROL AREA

LOCATION	DEPTH (CM)	CONCENTRATION (PPT)
Grid I	0-15.0	10
Grid I	0-15.0	25
Grid I	0-15.0	70
Grid I	0-15.0	70
Grid I	0-15.0	110
Grid I	0-15.0	710
Grid I	0-2.5	150
Grid I	2.5-5.0	160
Grid I	5.0-10.0	700
Grid I	10.0-15.0	44
Control	0-15.0	ND[a]
Control	0-15.0	ND
Control	0-15.0	ND
Control	0-15.0	ND

[a]Not detected at a lower detection limit of 6 ppt TCDD

TABLE 3. CONCENTRATION (PPT) OF TCDD IN LIVER AND PELT SAMPLES FROM BEACH MICE, PEROMYSCUS POLIONOTUS, COLLECTED FROM CONTROL AND TCDD-EXPOSED FIELD SITES, 1974

TREATMENT	SEX	LIVER	PELT
Control	Male	51	<40[a]
	Female	83	<40[a]
Grid I	Male	1,300	130
	Female	960	140

[a]Minimum level of detection.

The livers of both male and female mice from the control area also contained TCDD, but at a much lower level than those from Grid I, with the males having a TCDD level of 51 ppt and the females 83 ppt. For the males this was only 3.9% of the level found in the test animals and for the females only 8.6%. With the minimum level of detection at 40 ppt, TCDD was not detected on the pelts of either the control males or the control females.

No TCDD was found in the livers and pelts from beach mice dusted 10 times in a period of 28 days with alumina gel containing no TCDD. The animals dusted with alumina gel containing 2.5 ppb TCDD had detectable levels on their pelts of 45 ppt for males and 89 ppt for females. The pooled sample of liver tissue contained 125 ppt TCDD. (Due to the small amounts of liver tissue available, analysis by sex for TCDD in the liver was not possible.)

Body Weight and Organ Weight Analysis

The basic body weight and organ weight data for the field study are shown in Tables 4 and 5. An analysis of body weights per se was not attempted since the ages of the beach mice were not known and the animals could only be classified by sex and treatment.

The data were first examined using regression analysis followed by a two-tailed test of the normal distribution to determine whether the correlation coefficients differed significantly between the control and test groups. For this analysis, the animals were divided into groups according to treatment and sex, and were then examined for linear correlation, semi-logarithmic correlation, and double logarithmic

TABLE 4. BODY WEIGHTS AND ORGAN WEIGHTS OF CONTROL PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	BODY WT (GM)	LIVER WT (GM)	SPLEEN WT (MG)	ADRENAL WT (MG)	KIDNEY WT (MG)	HEART WT (MG)	LUNG WT (MG)
M	L-118	12.75	.530	20	14	174	105	102
M	L-148	14.65	.811	17	32	226	108	119
M	L-194	10.44	.580	16	17	174	84	94
M	L-230	12.62	.778	12	20	183	93	68
M	L-499	11.72	.726	15	28	211	90	110
M	L-841	11.70	.537	16	11	195	130	92
M	L-886	12.59	.495	14	23	207	96	112
M	L-917	12.66	.524	21	18	199	113	108
M	L-932	11.45	.548	20	21	171	100	96
F	L-322	10.23	.679	25	12	170	84	88
F	L-473	9.93	.730	30	26	168	64	75
F	L-661	16.40	.864	24	26	253	102	120
F	L-666	12.96	.831	24	20	195	94	106
F	L-671	11.61	.642	26	17	171	77	99
F	L-744	7.77	.303	14	12	125	53	82
	Male	12.29 ±1.17	.614 ±.122	16.78 ±3.03	20.44 ±6.58	193.33 ±19.22	102.11 ±13.87	100.11 ±15.05
	Female	11.48 ±2.97	.675 ±.201	23.83 ±5.31	18.83 ±6.34	180.33 ±42.20	79.0 ±18.35	95.0 ±16.61

TABLE 5. BODY WEIGHTS AND ORGAN WEIGHTS OF TREATED PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	BODY WT (GM)	LIVER WT (GM)	SPLEEN WT (MG)	ADRENAL WT (MG)	KIDNEY WT (MG)	HEART WT (MG)	LUNG WT (MG)
M	L-051	11.49	.824	29	30	201	113	103
M	L-249	10.06	.529	14	18	187	73	80
M	L-529	11.09	.635	9	22	174	84	81
M	L-555	10.05	.436	12	18	204	149	124
M	L-579	11.74	.797	45	22	191	70	85
M	L-611	13.63	.696	11	27	196	97	112
M	L-729	11.63	.750	35	27	204	82	84
M	L-751	9.24	1.017	17	10	203	90	78
M	L-805	12.25	.696	16	31	234	97	124
M	L-959	9.32	.725	37	15	168	80	95
F	L-009	13.49	.922	11	16	249	114	130
F	L-251	8.63	.493	17	10	147	91	82
F	L-538	16.32	1.044	55	24	241	108	82
F	L-558	9.46	.828	54	16	163	81	83
F	L-797	15.57	.926	17	19	216	111	90
	Male	11.05 ±1.39	.710 ±.160	22.5 ±12.84	22.00 ±6.83	196.2 ±18.32	93.5 ±23.27	96.6 ±18.08
	Female	12.69 ±3.50	.843 ±.210	30.8 ±21.78	17.00 ±5.10	203.2 ±46.00	101.0 ±14.30	93.4 ±20.73

correlation of the absolute organ weight to absolute body weight. The correlation coefficients were not significantly different at the 0.05 level.

The absolute organ weight data were then converted to display the organ weights as percent of body weight. These converted data are presented in Tables 6 and 7.

Using the Wilcoxon Rank Sum Test to examine the groups that have been separated according to treatment and sex, differences in the converted data for the kidney and liver were noted between the two male groups. However, when it was noted that the data from one animal (L-751) for the liver and kidney deviated from the mean by 2.5 or more standard deviations, the data were reexamined, omitting the data from that animal, and no significant differences were seen at the 0.05 level.

Again omitting the data from one animal (L-751), the organ weights were reexamined with an anlysis of covariance using the body weight as the covariate. At the 95 percent level of confidence, using this procedure of analysis, the only difference between exposed and controlled field groups was in liver weight. The exposed field group had a significantly greater liver weight than did the control group.

The initial body weight data for the beach mice used in the laboratory dusting study were compared with the final body weights in Table 8. Ignoring sex of animals, the data indicated that the control animals exhibited a slight weight gain during the 28-day study (+0.17 g) while the test group showed a slight decline (-0.45 g). Statistical analysis of the weight change using the Wilcoxon Rank Sum Test (p=0.05)

TABLE 6. ORGAN WEIGHTS, EXPRESSED AS PERCENT OF BODY WEIGHT, OF CONTROL PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	LIVER	SPLEEN	ADRENAL	KIDNEY	HEART	LUNG
M	L-118	4.16	0.16	0.11	1.36	0.82	0.80
M	L-148	5.54	0.12	0.22	1.54	0.74	0.81
M	L-194	5.56	0.15	0.16	1.67	0.80	0.90
M	L-230	6.16	0.10	0.16	1.45	0.74	0.54
M	L-499	6.19	0.13	0.24	1.80	0.77	0.94
M	L-841	4.59	0.14	0.09	1.67	1.11	0.79
M	L-886	3.93	0.11	0.18	1.64	0.76	0.89
M	L-917	4.14	0.17	0.14	1.57	0.89	0.85
M	L-932	4.79	0.17	0.18	1.49	0.87	0.84
F	L-322	6.64	0.24	0.12	1.66	0.82	0.86
F	L-473	7.35	0.30	0.26	1.69	0.64	0.76
F	L-661	5.27	0.15	0.16	1.54	0.62	0.73
F	L-666	6.41	0.19	0.15	1.50	0.73	0.82
F	L-671	5.53	0.22	0.15	1.47	0.66	0.85
F	L-744	3.90	0.18	0.15	1.61	0.68	1.06
	Male	5.01 ±0.88	0.14 ±0.03	0.16 ±0.05	1.58 ±0.13	0.83 ±0.12	0.82 ±0.12
	Female	5.85 ±1.22	0.21 ±0.05	0.16 ±0.05	1.58 ±0.09	0.69 ±0.07	0.85 ±0.12

TABLE 7. ORGAN WEIGHTS, EXPRESSED AS PERCENT OF BODY WEIGHT, OF TREATED PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	LIVER	SPLEEN	ADRENAL	KIDNEY	HEART	LUNG
M	L-051	7.17	0.25	0.26	1.75	0.98	0.90
M	L-249	5.26	0.14	0.18	1.86	0.73	0.80
M	L-529	5.73	0.08	0.20	1.57	0.76	0.73
M	L-555	4.34	0.12	0.18	2.03	1.48	1.23
M	L-579	6.79	0.38	0.19	1.63	0.60	0.72
M	L-611	5.11	0.08	0.20	1.44	0.71	0.82
M	L-729	6.45	0.30	0.23	1.75	0.71	0.72
M	L-751	11.01	0.18	0.11	2.20	0.97	0.84
M	L-805	5.68	0.13	0.25	1.91	0.79	1.01
M	L-959	7.78	0.40	0.16	1.80	0.86	1.02
F	L-009	6.83	0.08	0.12	1.85	0.85	0.96
F	L-251	5.71	0.20	0.12	1.70	1.05	0.95
F	L-538	6.40	0.34	0.15	1.48	0.66	0.50
F	L-558	8.75	0.57	0.17	1.72	0.86	0.88
F	L-797	5.95	0.11	0.12	1.39	0.71	0.58
	Male	6.53 ±1.88	0.21 ±0.12	0.20 ±0.04	1.80 ±0.22	0.86 ±0.25	0.88 ±0.16
	Female	6.73 ±1.21	0.26 ±0.20	0.14 ±0.02	1.63 ±0.19	0.83 ±0.15	0.77 ±0.22

TABLE 8. INITIAL AND FINAL BODY WEIGHTS OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING 2.5 PPB TCDD (TEST)[a]

CONTROL GROUP WEIGHTS (GRAMS)			TEST GROUP WEIGHTS (GRAMS)		
INITIAL	FINAL	DIFFERENCE	INITIAL	FINAL	DIFFERENCE
17.06	17.55	+0.44	12.69	12.07	-0.62
13.50	16.80	+3.30	16.10	15.72	-0.38
11.00	11.43	+0.43	13.12	12.77	-0.35
13.40	12.60	-0.80	17.15	18.02	+0.87
15.25	14.23	-1.02	13.71	13.65	-0.06
12.50	12.72	+0.22	14.48	13.20	-1.28
14.01	14.38	+0.37	14.90	15.57	+0.67
13.12	13.10	-0.02	12.36	11.78	-0.58
14.10	13.26	-0.84	14.03	12.61	-1.42
13.40	12.97	-0.43	16.00	14.94	-1.01
			13.90	13.77	-0.13
			15.25	14.12	-1.13

[a]Data on sex of animals are shown in Tables 9 and 10.

TABLE 9. BODY WEIGHTS AND ORGAN WEIGHTS OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL)

SEX	SPECIMEN #	BODY WT (GM)	LIVER WT (GM)	SPLEEN WT (MG)	ADRENAL WT (MG)	KIDNEY WT (MG)	HEART WT (MG)	LUNG WT (MG)
M	069	12.97	.718	14	27	190	158	118
M	323	14.38	.686	13	26	207	81	125
M	626	12.72	.610	10	22	186	75	95
M	628	13.26	.698	19	43	118	100	101
M	655	12.60	.577	10	26	199	115	95
M	669	13.10	.645	23	30	197	130	79
F	112	16.80	.980	24	49	255	112	106
F	274	14.23	.825	20	28	230	132	95
F	591	11.43	.606	14	46	201	92	80
F	696	17.55	.951	26	41	258	156	112
	Male	13.17 ±0.64	0.656 ±0.055	14.33 ±4.32	29.00 ±7.32	182.83 ±32.59	109.83 ±31.29	102.17 ±16.81
	Female	15.00 ±2.77	0.840 ±0.170	21.00 ±5.29	41.00 ±9.27	236.00 ±26.50	123.00 ±27.39	98.25 ±14.06

TABLE 10. BODY WEIGHTS AND ORGAN WEIGHTS OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING 2.5 PPB TCDD (TEST)

SEX	SPECIMEN #	BODY WT (GM)	LIVER WT (GM)	SPLEEN WT (MG)	ADRENAL WT (MG)	KIDNEY WT (MG)	HEART WT (MG)	LUNG WT (MG)
M	221	18.02	.790	25	42	225	156	123
M	296	13.20	.713	24	39	202	119	92
M	372	14.12	.779	22	27	226	116	109
M	446	13.65	.805	19	34	246	105	88
M	528	12.77	.542	20	32	189	122	90
M	742	15.57	.723	33	59	214	127	90
M	966	15.72	.953	37	25	226	144	107
F	054	13.77	.832	9	31	243	123	88
F	073	12.61	.751	14	28	219	101	112
F	224	12.07	.714	17	30	195	98	84
F	444	11.78	.593	17	20	196	117	80
F	641	14.99	.912	14	35	279	126	113
	Male	14.72 ±1.84	0.758 ±0.124	25.71 ±6.78	36.86 ±11.48	218.29 ±18.59	127.00 ±17.44	99.86 ±13.33
	Female	13.04 ±1.33	0.760 ±0.121	14.20 ±3.27	28.80 ±5.54	226.40 ±35.38	113.00 ±12.79	95.40 ±15.87

indicated no significant difference. No significant difference in weight change was found when the animals were compared according to sex.

The post-mortem body weight and organ weight data for the laboratory dusting study are shown in Tables 9 and 10.

For satistical analysis the organ weight and body weight data from the laboratory animals were also grouped according to treatment and sex before examination for linear correlation, semi-logarithmic correlation, and double logarithmic correlation of the absolute organ weight to absolute body weight. A two-tailed test of the normal distribution was used to determine whether correlation coefficients of control and treated groups differed significantly from each other. At the 0.05 level a significant difference was noted between the spleen weight to body weight coefficients of the control female and treated female beach mice.

The organ weight data from the laboratory study were also converted to be expressed as percent of body weight. These data are presented in Tables 11 and 12.

After separating the groups according to treatment and sex, significant differences attributable to treatment could be seen in spleen to body weight ratios for the control male and treated male groups ($p=0.05$). Sex differences were also noted in the data for kidney, liver, and spleen for the treated male/treated female, control male/control female, and treated male/treated female groups respectively.

Examination of the organ weight data with an analysis of covariance, using the body weight as the covariate, revealed none of the previously found differences. Indeed, this statistical analysis showed there were

TABLE 11. ORGAN WEIGHTS, EXPRESSED AS PERCENT OF BODY WEIGHT, OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL)

SEX	SPECIMEN #	LIVER	SPLEEN	ADRENAL	KIDNEY	HEART	LUNG
M	069	5.54	0.11	0.21	1.47	1.22	0.91
M	323	4.77	0.09	0.18	1.44	0.56	0.87
M	626	4.80	0.08	0.17	1.46	0.59	0.75
M	628	5.26	0.14	0.32	0.89	0.75	0.76
M	655	4.58	0.08	0.21	1.58	0.91	0.75
M	669	4.92	0.15	0.23	1.50	0.99	0.60
F	112	5.83	0.14	0.29	1.52	0.67	0.63
F	274	5.80	0.14	0.20	1.62	0.93	0.67
F	591	5.30	0.12	0.40	1.76	0.80	0.70
F	696	5.42	0.15	0.23	1.47	0.89	0.64
	Male	4.98 ±0.36	0.11 ±0.03	0.22 ±0.05	1.39 ±0.25	0.84 ±0.25	0.77 ±0.11
	Female	5.59 ±0.27	0.14 ±0.01	0.28 ±0.09	1.59 ±0.13	0.82 ±0.12	0.66 ±0.03

TABLE 12. ORGAN WEIGHTS, EXPRESSED AS PERCENT OF BODY WEIGHT, OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING 2.5 PPB TCDD (TEST)

SEX	SPECIMEN #	LIVER	SPLEEN	ADRENAL	KIDNEY	HEART	LUNG
M	221	4.38	0.14	0.23	1.25	0.87	0.68
M	296	5.40	0.18	0.30	1.53	0.90	0.70
M	372	5.52	0.16	0.19	1.60	0.82	0.77
M	446	5.90	0.14	0.25	1.80	0.77	0.64
M	528	4.24	0.16	0.25	1.48	0.96	0.70
M	742	4.64	0.21	0.38	1.37	0.82	0.58
M	966	6.06	0.24	0.16	1.44	0.92	0.68
F	054	6.04	0.07	0.23	1.76	0.89	0.64
F	073	5.96	0.11	0.22	1.74	0.80	0.89
F	224	5.92	0.14	0.25	1.62	0.81	0.70
F	444	5.03	0.14	0.17	1.66	0.99	0.68
F	641	6.08	0.09	0.23	1.86	0.84	0.75
	Male	5.16 ±0.74	0.18 ±0.04	0.25 ±0.07	1.50 ±0.18	0.87 ±0.07	0.68 ±0.06
	Female	5.81 ±0.44	0.11 ±0.03	0.22 ±0.03	1.73 ±0.09	0.87 ±0.08	0.73 ±0.10

no significant differences in the organ weights of the control and test groups in the laboratory dusting study ($p=0.05$).

Histopathology

The supporting histopathological studies were performed by the Veterinary Pathology Division, Armed Forces Institute of Pathology on both test and control mice with no distinction being made between the animals from the field study and the animals from the laboratory (dusting) study. A series of histological examinations were performed on the heart, lungs, trachea, salivary glands, thymus, liver, kidneys, stomach, pancreas, adrenals, large and small intestines, spleen, genital organs, bone, bone marrow, skin, and brain.

Initially, the tissues were examined on a random basis without the knowledge of whether the mouse was a control or test animal. All microscopic changes, including those interpreted as minor or insignificant, were recorded. Following the recording of all microscopic findings, the tissues were reexamined on a control and test basis. Results of both studies determined that the test and control mice could not be distinguished on a microscopic basis.

Significant lesions were found in only one mouse, a test mouse from the field study. The liver displayed moderately severe, multifocal, necrotizing hepatitis (Figure 3). Sections from the liver of this animal were stained from a variety of stains in attempts to identify an etiologic agent. Neither bacterial or funal organisms were demonstrated and the lesions were considered viral induced as they resembled the lesions seen in viral hepatitis of laboratory mice.

The gross lesions observed in the kidney of one test mouse from the field study proved to be severe ectasia of renal veins. Microscopically, the vascular dilatation was interpreted as being of little functional significance (Figure 4). All other lesions observed in both control and test mice were minor and insignificant and of the type normally observed when a large group of animals are examined at the microscopic level.

Hepatic Morphometric Analysis

The hepatic morphometric data for each animal in the field study are presented as mean values in Tables 13 and 14. Since morphometric analysis is concerned with the volume fraction of each structure in question or the ratio between the point count of that structure and the total count of the cytoplasm, and since the count for each structure could vary with cell size, only the total cytoplasm count was statistically analyzed for differences. Using the Wilcoxon Rank Sum Test to examine the total counts ($p=0.05$), no significant difference was seen between the control and treated field animals.

After the volume fraction was determined for each required structure of the photographed cells, the means were computed for each animal by sex and treatment and presented in Table 15. There were no significant differences between field control and field treated animals for any of the cellular structures in question ($p=0.05$).

The hepatic morphometric data for the laboratory study animals were treated the same as the data from the field study. The mean values are shown in Tables 16 and 17. After being separated according to sex and treatment. The total cytoplasm count (indicating cell size) showed

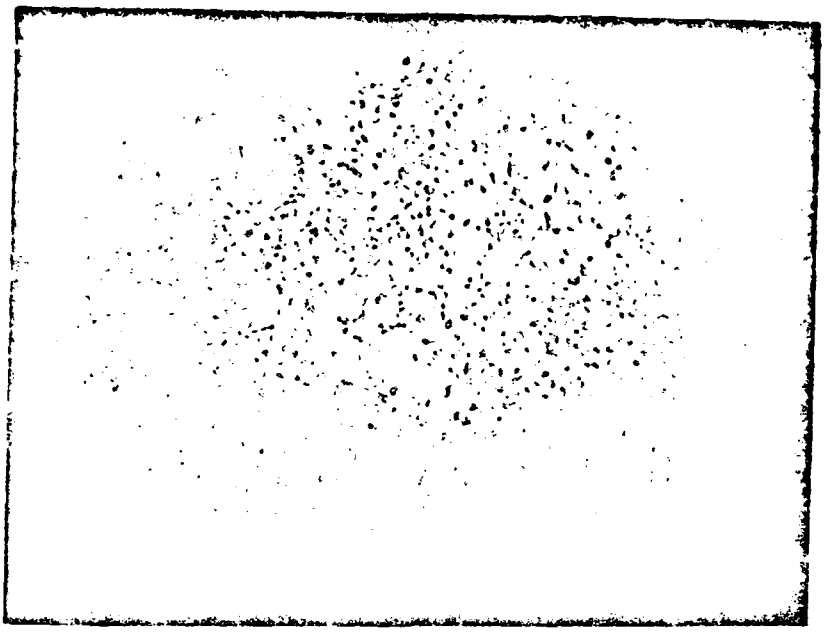

Figure 3. Acute Necrosis and Inflammation in the Liver of a Beach Mouse Captured from Grid I. Hematoxylin and Eosin.

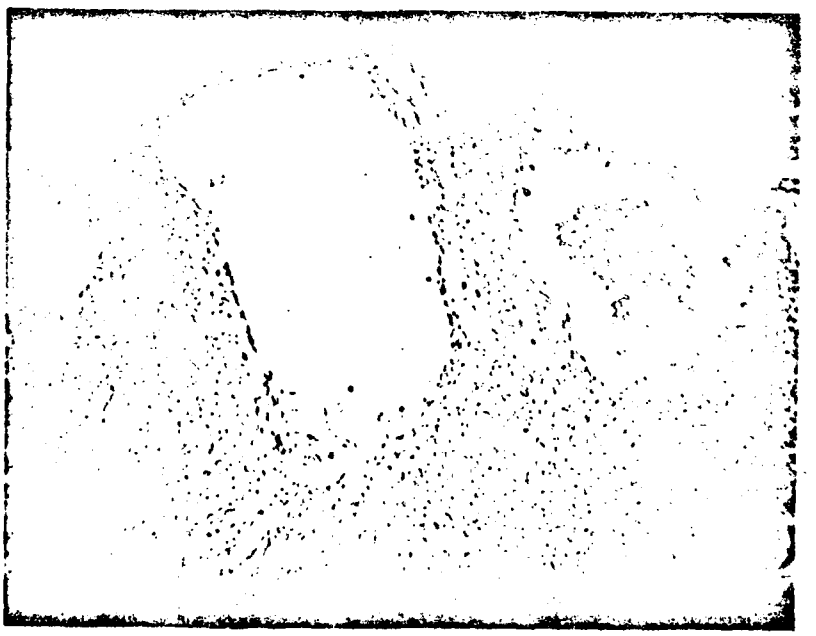

Figure 4. Microscopic Appearance of Venous Ectasia in the Kidney of a Beach Mouse Captured from Grid I. Hematoxylin and Eosin.

TABLE 13. HEPATIC MORPHOMETRIC DATA OF CONTROL PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	TOTAL COUNT	MITO COUNT	DAMAGED MITO COUNT	RER COUNT	SER COUNT
M	L-118	549.0	140.3	6.0	52.4	273.9
M	L-148	438.4	100.2	0	89.6	192.8
M	L-194	321.0	63.2	6.2	95.2	138.0
M	L-230	434.4	63.6	9.8	65.2	201.2
M	L-499	378.8	59.6	2.4	78.6	146.6
M	L-841	374.6	102.2	1.8	94.2	110.6
M	L-886	353.8	105.4	10.2	59.0	135.8
M	L-917	273.9	71.4	9.6	49.9	116.4
M	L-932	225.3	47.9	1.6	67.9	58.9
F	L-322	506.0	119.0	14.8	115.8	210.6
F	L-473	275.2	41.6	7.8	91.6	120.6
F	L-661	324.9	92.0	9.9	59.7	122.1
F	L-666	308.8	67.5	1.5	58.5	136.2
F	L-671	445.0	81.4	1.0	140.6	128.8
F	L-744	170.9	73.2	0.4	23.7	58.4
	Male	372.1 ±96.02	83.76 ±29.85	5.29 ±3.98	72.44 ±17.63	152.69 ±62.33
	Female	338.5 ±120.47	79.12 ±25.84	5.9 ±5.87	81.65 ±42.70	129.45 ±48.60

TABLE 14. HEPATIC MORPHOMETRIC DATA OF TREATED PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

SEX	SPECIMEN #	TOTAL COUNT	MITO COUNT	DAMAGED MITO COUNT	RER COUNT	SER COUNT
M	L-051	326.2	82.8	16.2	27.2	156.2
M	L-249	269.8	58.0	0.2	40.8	108.0
M	L-529	405.2	94.4	1.8	76.2	182.4
M	L-555	265.5	102.5	0	41.0	97.0
M	L-579	390.5	67.5	1.5	34.0	230.0
M	L-611	451.0	74.4	3.2	75.8	139.4
M	L-729	277.8	56.4	0	55.0	128.0
M	L-751	439.3	104.3	9.3	54.7	226.7
M	L-805	211.8	49.6	0.2	39.2	102.6
M	L-959	353.8	81.3	8.2	78.3	132.7
F	L-009	418.0	82.8	2.5	84.5	225.0
F	L-251	185.0	52.7	6.0	39.3	67.3
F	L-538	333.0	87.6	5.0	77.2	144.0
F	L-558	410.2	75.5	0	60.2	223.8
F	L-797	400.0	93.8	1.8	73.0	175.2
	Male	339.1 ±81.74	77.12 ±19.39	4.06 ±5.45	52.22 ±18.88	150.3 ±48.40
	Female	349.2 ±97.80	78.48 ±15.89	3.06 ±2.43	66.84 ±17.75	167.06 ±65.43

TABLE 15. HEPATIC MORPHOMETRIC DATA, EXPRESSED AS RATIOS, OF CONTROL AND TREATED PEROMYSCUS POLIONOTUS OBTAINED IN JUNE 1974, TEST AREA C-52A, EGLIN AFB, FLORIDA

LOCATION	SEX	SPECIMEN #	MITO/TOT	d.MITO/MITO	RER/TOT	SER/TOT	RER/SER
Control	M	L-118	.272	.044	.105	.481	.226
Control	M	L-148	.228	.001	.207	.436	.476
Control	M	L-194	.199	.075	.287	.432	.666
Control	M	L-230	.148	.150	.150	.490	.334
Control	M	L-499	.160	.040	.211	.377	.529
Control	M	L-841	.272	.017	.249	.299	.878
Control	M	L-886	.297	.088	.166	.385	.454
Control	M	L-917	.264	.151	.185	.421	.438
Control	M	L-932	.212	.032	.303	.267	1.258
Control	F	L-322	.228	.125	.232	.409	.578
Control	F	L-473	.150	.213	.340	.435	.837
Control	F	L-661	.286	.121	.182	.376	.487
Control	F	L-666	.223	.021	.182	.443	.419
Control	F	L-671	.192	.011	.313	.294	1.102
Control	F	L-744	.428	.005	.138	.343	.415
Treated	M	L-051	.255	.201	.083	.476	.176
Treated	M	L-249	.211	.002	.164	.410	.409
Treated	M	L-529	.234	.019	.187	.452	.424
Treated	M	L-555	.372	.001	.158	.376	.421
Treated	M	L-579	.178	.022	.084	.589	.143
Treated	M	L-611	.162	.059	.171	.304	.576
Treated	M	L-729	.198	.001	.199	.457	.438
Treated	M	L-751	.238	.082	.122	.524	.238
Treated	M	L-805	.231	.003	.180	.488	.389
Treated	M	L-959	.226	.067	.219	.386	.594
Treated	F	L-009	.201	.037	.213	.511	.435
Treated	F	L-251	.270	.111	.218	.358	.640
Treated	F	L-538	.258	.052	.234	.434	.550
Treated	F	L-558	.183	.001	.147	.549	.271
Treated	F	L-797	.242	.022	.197	.424	.489
Control Male			0.228 ±0.052	0.066 ±0.055	0.207 ±0.064	0.399 ±0.076	0.584 ±0.314
Control Female			0.251 ±0.098	0.083 ±0.084	0.231 ±0.080	0.383 ±0.058	0.640 ±0.275
Treated Male			0.230 ±0.057	0.046 ±0.062	0.157 ±0.046	0.446 ±0.081	0.381 ±0.153
Treated Female			0.231 ±0.037	0.045 ±0.042	0.202 ±0.033	0.455 ±0.075	0.477 ±0.138

TABLE 16. HEPATIC MORPHOMETRIC DATA OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL)

SEX	SPECIMEN #	TOTAL COUNT	MITO COUNT	DAMAGED MITO COUNT	RER COUNT	SER COUNT
M	069	386.0	84.2	10.2	71.8	131.4
M	323	445.6	115.2	7.2	57.4	181.4
M	626	455.0	137.0	44.4	62.8	183.0
M	628	359.4	91.4	16.8	49.6	126.8
M	655	524.4	112.6	35.2	84.4	229.4
M	669	386.2	110.2	46.6	57.4	140.2
F	112	400.0	74.2	15.0	47.2	155.8
F	274	280.8	69.8	13.3	39.5	107.3
F	591	376.6	95.2	27.4	55.8	150.2
F	696	477.8	124.6	30.0	75.0	155.6
	Male	426.10 ±60.87	108.43 ±18.76	26.73 ±17.50	63.90 ±12.43	165.37 ±39.86
	Female	383.80 ±81.16	90.95 ±25.02	21.42 ±8.50	54.38 ±15.28	142.22 ±23.43

TABLE 17. HEPATIC MORPHOMETRIC DATA OF PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING 2.5 PPB TCDD (TEST)

SEX	SPECI-MEN #	TOTAL COUNT	MITO COUNT	DAMAGED MITO COUNT	RER COUNT	SER COUNT
M	221	432.0	91.8	22.2	85.4	168.2
M	296	437.0	96.4	28.8	67.4	183.6
M	372	349.8	88.4	19.4	69.2	115.4
M	446	343.6	97.4	27.8	55.0	133.2
M	528	379.0	120.4	18.6	54.6	159.0
M	742	333.0	84.2	22.4	69.0	134.2
M	966	388.2	134.0	35.4	59.8	143.4
F	054	284.5	66.7	9.8	60.2	102.8
F	073	462.7	125.8	38.3	60.0	170.0
F	224	358.2	71.6	13.2	48.0	150.2
F	444	435.6	102.6	14.8	73.8	170.4
F	641	473.4	109.2	35.4	57.6	204.6
	Male	380.37 ±41.77	101.80 ±18.35	24.94 ±6.02	65.77 ±10.70	148.14 ±23.42
	Female	402.88 ±80.05	95.18 ±25.28	22.30 ±13.44	59.92 ±9.22	159.60 ±37.30

no significant difference between the control and treated laboratory animals. (As with the field animals, this was the only data, not expressed as ratios, analyzed statistically.) The mean volume fraction, or ratio for each required cellular structure of each animal in the laboratory dusting study are shown in Table 18.

The volume fractions or ratios from treated laboratory animals were compared with those from control animals using the Wilcoxon Rank Sum Test ($p=0.05$). No significant differences were noted between animals dusted with alumina gel containing no TCDD (control) and animals dusted with alumina gel containing 2.5 ppb TCDD (test).

General Cellular Observations

Concentric membrane arrays (myelin figures) mitotic figures, and multinucleated hepatocytes were not observed during viewing of the tissue for photograph. However, occasional binucleated cells were seen and two basic types of parenchymal cells were differentiated on the basis of staining intensity.

TABLE 18. HEPATIC MORPHOMETRIC DATA, EXPRESSED AS RATIOS, OF *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL) OR WITH ALUMINA GEL CONTAINING 2.5 PPB TCDD (TEST)

TREATMENT	SEX	SPECIMEN #	MITO/TOT	d.MITO/MITO	RER/TOT	SER/TOT	RER/SER
Control	M	069	.219	.146	.189	.340	.566
Control	M	323	.257	.083	.128	.410	.323
Control	M	626	.295	.294	.142	.400	.359
Control	M	628	.257	.139	.138	.350	.403
Control	M	655	.210	.269	.162	.436	.382
Control	M	669	.278	.349	.150	.364	.418
Control	F	112	.183	.226	.122	.394	.328
Control	F	274	.253	.174	.151	.374	.419
Control	F	591	.256	.286	.149	.400	.371
Control	F	696	.264	.244	.155	.324	.471
Treated	M	221	.216	.249	.197	.392	.510
Treated	M	296	.222	.278	.154	.423	.367
Treated	M	372	.252	.231	.200	.328	.614
Treated	M	446	.281	.285	.160	.383	.420
Treated	M	528	.318	.153	.143	.421	.346
Treated	M	742	.254	.213	.208	.396	.540
Treated	M	966	.340	.199	.155	.369	.422
Treated	F	054	.231	.119	.215	.367	.586
Treated	F	073	.266	.298	.136	.370	.369
Treated	F	224	.200	.175	.135	.423	.320
Treated	F	444	.238	.146	.170	.394	.431
Treated	F	641	.234	.321	.123	.425	.293
Control Male			0.253 ±0.033	0.213 ±0.105	0.152 ±0.022	0.383 ±0.038	0.408 ±0.084
Control Female			0.239 ±0.038	0.232 ±0.046	0.144 ±0.015	0.373 ±0.034	0.397 ±0.062
Treated Male			0.269 ±0.047	0.230 ±0.046	0.174 ±0.027	0.387 ±0.033	0.460 ±0.098
Treated Female			0.234 ±0.023	0.212 ±0.092	0.156 ±0.037	0.396 ±0.028	0.400 ±0.117

DISCUSSION

A factor of concern in interpreting the data was the sample size for both the field study and the laboratory study. The number of beach mice in each group, when separated by sex and treatment, ranged from five to ten in the field study and from four to seven in the laboratory study. In such small samples the deviation of one individual will strongly influence the data for the entire group. For this reason, caution must be used in the interpretation of the results.

Field Study

The soil samples from the test area displayed wide fluctuations in TCDD concentrations, probably as the result of unequal distribution of the herbicide during aerial dissemination. Three major flight paths intersected at Grid I and the soil samples were taken from areas thought to be on the flight paths. However, if the samples were obtained from an area outside the flight paths or from the intersection of all three flight paths, the TCDD levels would be expected to vary considerably. Nevertheless, analysis of the soil samples did show that the beach mice from Grid I were exposed to concentrations of TCDD up to 710 parts per trillion (ppt) in the soil. In contrast, the soil from the control areas did not contain TCDD at a minimum detection level of six ppt and therefore did not provide a source of exposure for the control animals. Since the seed samples from Grid I did not contain TCDD at a minimum detection level of one ppt, seeds from Grid I were probably not a source of TCDD.

The mice continually contaminated themselves with soil containing TCDD by repeated movement in and out of their burrows. It was observed

that the mice plug their burrows with about 15 cm of soil after they enter and then must burrow through this plug when they exit the tunnel. This recurrent burrowing activity in increased exposure to the contaminated soil. The levels in the pelt samples from mice trapped on Grid I confirm this method of contact. In contrast, TCDD was not detected in pelt samples from control animals.

Since the seeds from Grid I were probably not a source of TCDD and the contaminated soil was confirmed as a source of contact, there were no data from this study to support biomagnification of TCDD. However, the level of TCDD detected in the livers of beach mice collected from Grid I confirms uptake by the animals and substantiates bioaccumulation by the liver. In general, levels of TCDD in the livers were somewhat greater than the most concentrated zones of TCDD in the soil.

In the years 1962 through 1964, enough TCDD was applied to Grid I to accumulate to the concentration of 12,267 ppt in the top 15 cm of the soil (43). By 1974 the level had declined about 94 percent to approximately 700 ppt. This level, although far greater than the estimated 0.1 ppt concentration in the soil after normal application of the herbicide 2,4,5-T (19), is much less than that normally used in laboratory experiements (1,7,11,17,18,21,22,25,27,29). Although the beach mice were exposed to soil levels of TCDD as high as 700 ppt, it is highly doubtful that the level ingested through grooming would even approach the levels given to animals via gavage in laboratory experiments; consequently, the accumulation of TCDD in the liver was much less than that reported in laboratory studies.

Kociba et al. (22), in a chronic, two year study showed that rats given 0.01 µg TCDD/kg/day had an average TCDD content of 5100 ppt in

the liver. Rats given 0.001 µg TCDD/kg/day had an average of 540 ppt in the liver. The livers from beach mice collected from Grid I in this study had a TCDD content of 1300 ppt for males and 960 ppt for females. Extrapolation of the data would then give the beach mice a daily TCDD intake dose of approximately 0.0012 µg/kg. Although extrapolation between species is not always advisable, Fries and Marrow (8) did state that total retention of TCDD was closely related to total intake.

TCDD was also found in the livers of the beach mice collected from the control area, although at a much lower level. The presence of TCDD in these pooled samples may have been due to high levels in one or more mice that could have migrated from the test area to the control area. A previous trapping study in this area (42) reported the longest random travel distance observed to be slightly over 900 meters. A travel distance of this magnitude was considered rare but could account for the presence of TCDD in the control animals. Nevertheless, even though the levels in the control mice were low compared to the levels in the test animals, the use of these mice as true controls must be viewed with caution.

Satistically significant differences in organ weight to body weight ratios were noted between control and exposed beach mice. The increase in liver weight found in this study is in agreement with other investigators (8,10,13,21,25,27,28,29,37); however, the lack of additional changes can be explained only by the level of exposure, which is considerably lower than in these experiments. Kociba et al. (22) found changes in liver and thymus weights in rats given 0.1 or 0.01 µg TCDD/kg/day for a two year period but no change in organ weights due to treatment with 0.001 µg TCDD/kg/day. With an exposure rate of approximately 0.0012 µg TCDD/kg/day,

the exposed mice in this study could be expected to display data falling between the two lower exposure groups of the chronic study by Kociba et al. (22). This, in fact, was the case with all the data reported in this field study.

The histopathological examination of the field animals affirmed the absence of significant differences between the beach mice taken from Grid I and those taken from the control area. Except for one report of viral hepatitis and one of renal vein ectasia, all lesions were of the minor or insignificant type normally observed in microscopic surveys of large numbers of field animals. Neither of the more serious lesions observed were considered to result from exposure to TCDD. This is in agreement with investigators using comparable exposure levels (22).

The binucleated cells observed during electron microscope photography were considered normal since two nuclei have been reported in 25 percent of hepatic parenchymal cells (41). The appearance of two types of parenchymal cells differing in electron density has not been fully explained (1) but may represent a transition between parenchymal and ductal cells as Hampton suggests (12). Kociba et al. (22) observed both multinucleated and swollen hepatocytes in groups of rats given 0.1 or 0.01 µg TCDD/kg/day while the group given 0.001 µg/TCDD/kg/day displayed neither of these findings. No mention was made by these investigators of parenchymal cells differing in staining intensity.

The lower exposure level seen in this study, although much higher than that anticipated in an environment following normal herbicide application (19), may account for the absence of histopathological and ultrastructural changes that were seen in other experiments with

TCDD (7,11,18,21,22,28). The results of the chronic toxicity study on TCDD in rats by Kociba et al. (22) substantiate a lack of adverse effects at such a low dose level.

The lack of adverse effects from TCDD seen in mice from the test area may indicate the presence of some mechanism for physiological adaptation not necessarily present in the mice from the control area. Berry (2) has shown that mice from neighboring populations separated by distances of one to 2.5 km may differ considerably in their genetic composition. Since the distance separating the control and test areas falls within this range, genetic variation may be considered as an explanation. Indeed, several investigators (26,31,32,33) have shown that certain inbred strains of mice are nonresponsive in the detoxification of TCDD. To determine if this is indeed the situation with these beach mice would require a much more exhaustive experiment beyond the scope of this study.

Laboratory Study

The laboratory dusting study confirmed ingestion during grooming as a possible method of contamination of the beach mice livers. Although the TCDD levels in the liver and pelt samples from the treated animals in the dusting study were not as high as from mice collected from the test area, TCDD was not detected in samples from the laboratory control animals, giving a clear treated/control comparison. The relatively short exposure time (28 days) was probably responsible for the laboratory treated animals having lower TCDD levels than the field treated animals.

The findings of this dusting study are in agreement with those reported by Kociba et al. (22) in the group of animals given the lowest

dose of TCDD. The one exception is in spleen weight as compared to terminal body weight. An increase in spleen weight was found in males and a decrease was found in females dusted with TCDD. Although histopathological examination of the spleens, as well as of the other organs, failed to support any differences between the control and test animals, the change in spleen weight tends to agree with previous investigators (11,13,35,37,43) who suggest that the spleen may be the most sensitive organ by which to assess exposure to TCDD. While these investigators agree in a loss of spleen weight with exposure to TCDD (11,37) there is some disagreement on whether the male or the female is more sensitive (13,35). However, no explanation is given for the sex difference in sensitivity.

The 125 ppt TCDD found in the livers of the treated animals of this study falls far short of the 540 ppt TCDD in the livers of rats given 0.001 µg TCDD/kg/day by Kociba et al. (22), a dose level that caused no cellular effects considered to be of any toxicologic significance and within the limits of variation seen in the controls. Although the actual oral dose in this dusting study could not be determined, it was probably well below the 0.001 µg TCDD/kg level. The liver TCDD level of 125 ppt associated with this apparent low dose level resulted in histopathological findings and hepatic morphometric data which showed no significant differences between the control and treated animals.

Again, as in the field study, binucleated cells were observed but were considered normal. Light and dark staining cells were also noted but their significance could not be determined.

Since the dose level of TCDD in this study could not be determined, it is difficult to compare the results from the laboratory dusting study with those presented by other investigators. However, this study does demonstrate a possible method of contamination of the beach mice livers.

Methods

Previous investigators such as Weibel (40) have incorporated computer processing and stereological techniques to evaluate data and determine actual volumes of cell organelles. Buchanan (3), however, modified these techniques to determine relative values rather than absolute. It is this modified stereological technique that is used in the present study to compare cellular ultrastructure of control and treated groups.

These stereological techniques, also known as morphometric analysis or morphometry, have not been applied in a quantitative assessment of TCDD effects prior to this study (1,7,11,17,18,22,23, 27,28,29). Therefore, this study is the first to present data derived from actual measurements of TCDD effects on ultrastructural hepatic morphology rather than from microscopic observations and estimations.

CONCLUSIONS

The results of this study indicate that TCDD persisted for long periods of time in the soil of Test Area C-52A, Eglin Air Force Base, Florida. Soil samples taken from the 0.4 km^2 of Grid I confirm that leaching does not occur and that the TCDD remaining in the soil after 10 years is stratified within the top 0-15 cm of the soil. Persistence of the TCDD in the soil is thought to be related to the massive application rates rather than to the absence of chemical or biological degradation.

Although the levels of TCDD in the livers are slightly greater than those found in the soil, TCDD was not detected in the portion of the food chain consisting of seeds. The laboratory dusting study confirms, however, that ingestion of TCDD can occur as a result of body contact and subsequent grooming.

The results of this study indicate no significant ultrastructural changes in hepatic parenchymal cells in response to long term, low level exposure to TCDD (field), or in response to short term, low level exposure to TCDD (laboratory). The levels of TCDD encountered in this study, up to approximately 700 ppt in the soil and an average of approimately 1,000 ppt in the livers, are much less than those normally found in most laboratory experiments, but far greater than the estimated concentrations in the environment, or in animal tissues after normal application of the herbicide 2,4,5-T.

In addition, this study demonstrates the application of the analytical technique of stereology to field studies of toxicity. The

modified technique, as used in this study, combined with tissue processing found in many modern pathology laboratories can produce usable data in 36 to 48 hours, rendering sterology a possible tool for characterizing quantitative cellular responses to injury.

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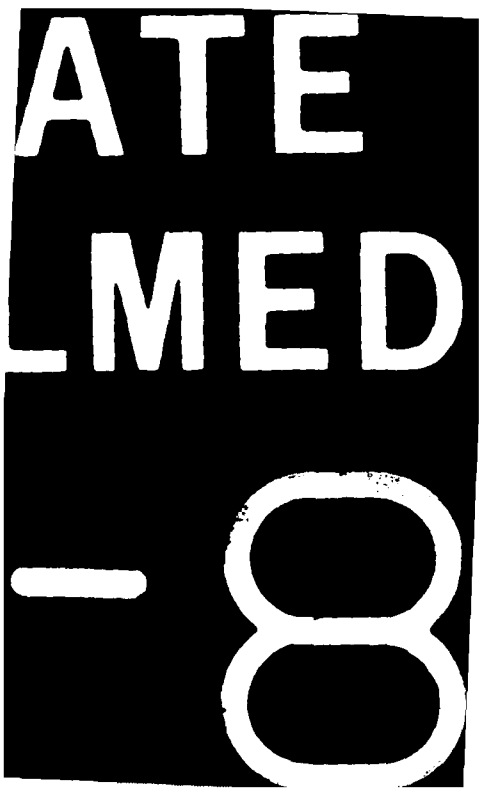
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